Microbial Degradation of Polyethylene Carry Bags of Low Density

A synopsis

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INTRODUCTION

Plastic is a broad name given to all different polymers which have high molecular weight. Plastic is a material that can be heated and molded in any shape, the word plastic comes from the Greek word *plastikos*. Different kinds of plastic find a number of applications in virtually every corner of everyday life from paper clips to aero planes. Plastic has replaced many traditional materials such as metal, wood, stone, leather, paper etc. and is used in food, clothing, toys, container, transportation, packing, construction, medical, recreational industries, automobiles and furniture etc. (Balasubramanian, et al. 2010)**.** Because of its low cost processing, great strength, flexibility, light weight, imperviousness to water, versatility and also great stability. Mostly used plastic are Polyethylene Terephthalate (PET), Polyetylene(PE), Nylons, Polybutylene, Terephthalate, Poly Propylene(PP), Polystyrene(PS), Polyvinyl Chloride(PVC) and Polyurethane (PU) (Rivard, et al. 1995). Among all the plastic forms used Polyethylene (PE) alone account 64% of total plastic production which are discarded within a very short time so a visible portion of municipal waste is polythene (Premraj and Dobley 2005). Polythene is largely used in packaging material, milk pouch, plastic bags, water bottles, disposable articles, and garbage container etc. and creates a various types of environmental problems (Krup, *et al.* 1992).

HISTORY OF POLYTHENE: Polythene was first produced by high pressure process in Britain 1930 by Imperial Chemical Industries (ICI). Reginald Gibson & Eric Fawcett discovered that ethylene gas could be converted into a white solid by heating it at very high pressure in presence of minute quantities of oxygen. Polythene is derived from either modifying gas (a methane, ethane, propane mix) or from the catalytic cracking of crude oil. To obtain highly purified polythene, it is piped directly from the refinery to a separate polymerization plant. Under optimal conditions of pressure, temperature and catalysts, the double bond of the ethylene monomer repeat units ranges from 1000 to 10,000 (molecular weight ranges from 28000g/mol to 280,000g/mol).

Polythene has become technologically important since 1940s as it has replaced glass, wood, masonry and other constructional materials and even metals. It also finds a wide industrial commercial and domestic application (Ambikadevi, et al. 2014).The invention of synthetic polymer (polythene) from crude oil seems as miracle in Chemistry and in Material Sciences, and paved the way to the production of one of the most versatile group of material ever produced. (Sivan 2011).

TYPES OF POLYTHENE: Polythene is a synthetic polymer having high molecular weight and high hydrophobic level. Polyethylene (PE) is the simplest hydrocarbon polymer produced by combination of the monomer molecules having [CH2-CH2]-n structure. Commercially, it is produced from ethylene (Myer 2002). Polyethylene is categorized into several different categories on the bases of its density and branching, such as Ultra high molecular weight polyethylene (UHMWPE); Ultra low molecular weight polyethylene (ULMWPE or PE-WAX); High molecular weight polyethylene (HMWPE); High density polyethylene (HDPE); High density cross-linked polyethylene (HDXLPE);Cross-linked polyethylene (PEX or XLPE); Medium density polyethylene (MDPE); P Linear low density polyethylene (LLDPE); Low density polyethylene (LDPE); Very low density polyethylene (VLDPE); Ethylene vinyl acetate co-polymer, Ethylene ionomers (Brydson 1999). Among all the types of polyethylene mainly three types are frequently used i.e. high density polyethylene (HDPE), low density polyethylene (LDPE), and linear low-density polyethylene (LLDPE).

EFFECT OF POLYTHENE: Increased consumption of plastic worldwide generates about 57 million tons of plastic waste annually. (Statistics of Foreign Trade of India, March 2001 DGFT, GOI). In India consumption of more than 12 million tonnes of plastic products was expected till 2012(Annual report by CPCB 2008). Degradation of plastic waste is very slow that’s why the accumulation of plastic waste is increasing day-by-day and causing dangerous environmental problems in land, air and water. This is posing ecological threat to flora and fauna and to global

ecology. Another cause of polythene pollution is absence of efficient method for safe disposal and illegal or inappropriate dumping of domestic and industrial refuse in environment.

Around the world almost a million tons of plastic waste is produced every day. About half of this eventually goes into the landfill, while the rest are deposited on the road side by wind and carried into rivers and in seas (Crystal 2014). When the plastic products are mixed in the soil and accumulate continuously, it affects the nutrients and water absorption by the crops and reducing their output. Littering of polythene also reduces the rate of rain water percolation (Pramilla, et al. 2012). In marine environment plastic pollution contribute about 60-80%, because plastic waste generated by human activity finally enter into the marine water through rivers, canals/channels and municipal drainages (Derraik 2002). In coastal areas about one million marine animals are dying due to their intestinal blockage. Plastic product accidentally ingested by animals cause death of animals and enters into the food chain (Secchi *et al.,* 1999). Many of these compounds can undergo significant biomagnifications and may pose a direct threat to human health. Toxic agent present in plastic are associated with many health problems, including developmental impairment, cancer, endocrine disruption, neurobehavioral changes, arthritis, breast cancer, diabetes etc.

DISPOSAL OF POLYTHENE: There are mainly three methods for plastic waste disposal : -

1. Burrying in Landfill- The first drawback associated with disposal of plastic waste is the fact that landfill facilities occupy space that could be utilized for more productive means, such as agriculture and second problem is slow degradability as this means the occupied land is unavailable for long periods of time (Zhang, et al. 2004). Plastic component of landfill waste have been shown to persist for more than 20 years. This is due to the limited availability of oxygen in landfill, plastic debris in landfill also act as a source for a number of secondary environmental pollutant such as volatile organics, benzene, toluene, xylene, ethylene benzenes, and trimethyl benzene, releasing gases and are contained in leachate (Tensel and Yildiz 2011).

2. Incineration- It burns of the plastic waste completely but at the same time causes heavy toxic fumes that are released into the environment causing air pollution for examples polycyclic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), heavy metals , toxic carbon and oxygen-based free radicals (Astrup, *et al*. 2009).
3. Recycling- This process address the environmental shortcomings of both landfill and incineration but it is time consuming and costly. A small part of the plastics can be recycled and the remaining goes to the burial sites. It is also an inefficient process. The presence of additives and impurities can complicate the recycling procedure and decrease both the yield and quantity of the recovered product (Valanvannidis, et al. 2008).

In last 10 years, several biodegradable plastics have been introduced in the market. However, none of them is efficiently biodegraded in landfill, for this reason none of the product has gained widespread use (Scott 1999). Scientist look forward for an eco friendly and cost effective way to degrade the plastic in the environment. Biodegradation is an attractive option for environment friendly and efficient disposal of plastic waste. It is a promising alternative to current practices for waste disposal as it is a low cost process, potentially much more efficient and does not produce secondary pollutant.

Biodegradation of polythene is chemical degradation of polythene by the naturally occurring microorganism such as bacteria, fungi (Weiland,*et al*.1995**).** Biodegradation of polythene can be achieved by two process i.e. aerobic biodegradation, which occur in presence of oxygen and the end product is CO_2 and anaerobic biodegradation, which occur in absence of oxygen and the end product is CH_4. In landfill and sediments, plastic are degraded anaerobically while in composite site and soil aerobic biodegradation takes place. Biodegradation leads to the production of water, carbon dioxide and methane as end products i.e. mineralization (Steven 2002). Microorganisms are successfully used to degrade plastics because of its excellent adherence and colonization properties. Once established on surface of polythene, microorganisms cover the whole area by forming mycelia mat or colonies and utilize this synthetic polymer as a carbon source and transforming the complex polymer into

simpler one. Microorganisms are capable of producing many polythene degrading enzymes in different quantity. Microbes attached to the surface of polythene, with the help of their enzymes can grow on the polythene by utilizing them and degrade the polythene by the process of mineralization and depolymerization.

BIODEGRADATION OF PLASTIC BY MICROORGANISM (bacteria & fungi): There are many reports of microorganism mediated biodegradation of polymer (polythene). Polythene having high molecular weight is a stable synthetic polymer and cannot be easily degraded by microorganism. Degradation of polymer can be achieved by modifying the polymer.

Polythene blended with biodegradable additive: Biodegradable additives include starch, cellulose, chitin etc. Biodegradation of polythene has been studied since nineties. Polythene blended with 6% starch is degraded by fungi *Phanerochaete chrysosporium* (Lee, et al. 1990). In 1992 Pometto *et al*. studied the degradation of heat treated polythene blended with starch and degraded *by Streptomyces viridosporous T7A, S. badius 252 and S. setonii 75vi2*. In 1997 Fourst *et al.* have reported the biodegradation of LDPE blend with cellulose by common fungi. El-Shafei, *et al*. 1998 reported the biodegradation of disposable polythene by fungi and *Streptomyces species*.

Polythene containing pro-oxidant: Prooxidants are generally transition metal including Fe, Co and Mn. Polythene containing prooxidant formed biofilm on its surface (Kounty *et al.* 2010), Fontanella *et al*., 2010) compared the biodegradability of various polythene films with a balanced content of antioxidant and prooxidant i.e. (manganese and iron or manganese, iron and cobalt) inoculated with *Rhodococcus rhodochrous* on mineral medium during 180 days.

UV and Thermally treated polythene: 60Hrs UV treated LDPE showed a weight loss of 6.2%more degradation compared to untreated LDPE with bacteria *Bravebacillus borstelensis* for 30 days (Hadad, *et al*. 2005) UV irradiated LDPE for 500 hrs enhanced the growth with fungi *Penicillium simlicissimum* YK (Yamada, et al. 2001)Thermally treated LDPE at 150^0C for 120 hrs showed

increase in carbonyl index by 23% when inoculated with fungi *P. chrysosporium* (Sepulveda, et al. 1999) LDPE and HDPE are thermally treated at 180^0C for 10 days were utilized by marine bacteria *Bacillus sphericus, Bacillus cereus, Brevundimonas vesicularies and Curtobacterium flaccumfaciens* (Sudhakar, et al. 2008).

Consortium of microorganism: Consortium of four fungi *Aspergillus niger, Penicillium funiculosum, Paecilomyces variotii and Gliocladium virens was* grown on thermally oxidized PE with Cobalt in agar medium (Weiland, *et al.* 1995). Mixed culture of fungi and bacteria (*Penicillum frequentus* and *Bacillus mycoides)* was found to grow on degradable polythene and weight of polythene was reduced by 7% whereas single culture of the same microorganism showed 0.50% reduction in weight (Seneviratne *et al.*2006).Consortium of bacterial strain *Microbacterium species* strainMK3, *Pseudomonas putida* strain MK4, *Bacterium* Te68R strain PN12, with nanoparticles Nanobarium titanate(NBT) yielded high percentage of degradation as compared to single bacterial strain (Kapri,*et al.,* 2010). In-vitro biodegradation of LDPE containing nanoparticles fullerene-60 was carried out by bacterial consortium *Pseudomonas aeruginosa strain PS1, Pseudomonas putida strain PW1, Pseudomonas aeruginosa strain C1*.

Recent researches have revealed that some microbial strain are capable of degrading polythene without any pretreatment and additive. These include the actinomycete *Rhodococcus ruber* strain C208 (Gilan *et al.* 2004). *Brevibacillus bostelensis* strain 707 is also capable of degrading standard polythene (Hadad,*et al.,* 2005). (Raaman, et al. 2012) focused 12% degradation of polythene within one month by the fungi *Aspergillus japonicas* and 8% by *A. niger*. In 2014 Das & Kumar reported LDPE degradation by 4 species of *Aspergillus* and 1 species of *Fusarium* within 60 days of exposure and concluded that *Fusarium species* have more potential than *Aspergillus species*.20% biodegradation of LDPE in vivo condition for 120 days resulted with Pseudomonas *aeruginosa* (Kyaw *et al*. 2012). Watanabe eot al. in 2009 isolated and identified 3 types of LDPE degrading

microbes i.e. *Bacillus circulans, B. brevius and B.sphaecicus* by soil burial method. LDPE is also degraded by some marine bacteria studied by (Sindhuja, *et al.* 2011).

The maximum 61.0% of polythene degradation by *Microbacterium paraoxydans* in terms of Fourier Transform Infrared coupled Attenuated Total Reflectance (FTIR-ATR) was recorded within two months by Rajandas *et al*., 2012.But degradation of polythene in terms of weight loss was recorded as 47.2% after 3 months of incubation with the *Aspergillus oryzae* by Aswale et al. 2010. 50% weight loss of the polythene by fungus, *Phanerochaete chrysosporium* after 8 month of regular shaking at room temperature with pH 4 is reported by Konduri *et al.*, 2011. El-Shafei *et al.*, 1998 reported gain in the polythene weight after cultivation of the microbes on the polythene, incubated at regular shaking for one month at 30^0C.

Low density polyethylene (LDPE) is one of the polymers that are nearly impossible to be degraded fully in faster way. Biodegradation is a promising method of solving this environmental issue. With this aim work will be planned to identify and isolate potent low density polyethylene (LDPE) degrading microbes from plastic waste, and subsequently study the degradation with various supporting parameters such as pretreatment by UV radiation, thermal treatment, use of biodegradable additive (starch and cellulose) and mixing of prooxidant in polythene which will enhance the biodegradability of polythene.

REVIEW OF LITERATURE

Work in India: There are many groups which are working on degradation of polythene in India. Some of them are listed as follows:-

Kathiresan (2003) proved that mangrove soil is rich in micro organism and is capable of degrading of polythene and plastic cups. He isolated microbes associated with the degradation of polythene and plastic cups and identified them as bacteria *Bacillus species, Staphylococcus species Streptococcus species, Diplococcus species* and *Micrococcus species , Moraxella species* and *Pseudomonas species and* Fungi including *Aspergillus niger, A. ornatus, A. cremeus, A. flavus, A. candidus, A.glaucus, A.nidulans.* Efficiency of the microbial degradation of plastic and polythene was analysed in shaker flask cultures. Among the fungal species *Aspergilus glaucus* degraded 28.80% of polythene and 7.26% of plastic cup in one month period and bacterial species *Pseudomonas species* degraded 20.5% of polythene and 8.16% of plastics in one month. An experiment was also designed for degrading the polythene bags and plastic cups, under the soil condition of two mangrove species i.e. *Rhizophora species* and *Avicennia species* and degradation was analyzed 2,4,6,9 months of incubation in soil.

Vijaya *et al.* (2007) studied the biodegradation of polyethylene in the natural environment. The study was carried out in two stages. In the first stage, degradation of polythene carry bags and cups were analyzed in lab condition. In the second stage the selected carry bags were buried in the compost soil along with municipal solid waste in order to facilitate biodegradation of plastic. Plastic carry bags of varying thickness, considered to be non biodegradable were also used in the study. Degradation of plastic by microorganisms was measured by the ASTM (American Society for Testing and Material) D882 and D5988 -96 standard loss in weight and reduction in the tensile strength of plastic films after composting with municipal solid waste for specific period of time were taken as a criteria of biodegradation of plastic films. The predominant microbial species identified from the sample of polythene bags and plastic cup *were Bacillus species, Staphylococcus species, Streptococcus species,*

Diplococcus species, Micrococcus species, Pseudomonas species and Moraxella species. Among the identified fungal strain *Aspergillus niger, A. ornatus, A.nidulans, A.cremeus, A. flavus, A.candidus and A. glauca*s were the predominant species.

Balasubramanian, *et al.* (2010) assessed the degradation of High density polythene (HDPE) by bacteria isolated from plastic waste dump site of Gulf of Mannar by enrichment procedure. Rationally they isolated 15 bacterial strains from GMB1 to GMB15. Out of 15 only 2 GMB5and GMB7 were selected based on degradation efficacy for further studies. The isolated bacterial strains were identified as *Arthrobacter species* and *Pseudomonas species*. Weight loss of HDPE by *Arthrobacter species* and *Pseudomonas species* was 12% & 15% after 30 days of incubation. BATH assay was done for identification of the cell surface hydrophobicity. Both protein content of the biofilm and flourescein diacetate hydrolysis were used to test the viability and protein density of the biomass. FTIR spectrum showed that Ester carbonyl bond index (ECBI), Keto carbonyl bond index(KCBI) and Vinyl bond index(VBI) were increased, indicating changes in functional group confirming the biodegradation.

Nanda *et al.* (2010) evaluated the biodegradability of natural and synthetic polythene by three different *Pseudomonas species* isolated from various locations i.e. domestic waste disposal site dumped with household garbage and vegetable waste, soil from textile effluent drainage site and soil dumped with sewage, sludge, respectively in laboratory condition by a pure culture shake-flask incubation method. The percentage of biodegradation was evaluated by comparing the initial and final dry weight of polythene before and after incubation in their respective culture media. Among all the treatments *Pseudomonas* from sewage sludge dump was found to degrade both natural and synthetic polythene very efficiently with 46.2%. *Pseudomonas* with natural polythene and 29.1% for *Pseudomonas* with synthetic polythene.

Pramilla, *et al.* (2011) isolated fungi from sea water for the degradation of LDPE. Some preliminary experiments proved that LDPE can be biodegradable if the right micro organism is isolated .It was

proved that the hydrophobic LDPE film can act as substrate for the micro organism which formed a bio-film on the LDPE film. The isolated fungi grew on minimal medium containing LDPE in the powdered form as the carbon source even without any nitrogen source. Sturm test was conducted for calculating CO_2 which evolved after degradation of polythene and very good result was obtained. Isolated fungi identified as *Aspergillus versicolor* and *Aspergillus species* showed a good degradation rate of 77% and 8% respectively.

Singh *et al.* (2012) checked the biodegradability of Low density polythene (LDPE) by *Aspergillus fumigates* and *Penicillium species* in lab condition. After 30 days of incubation, growth of both the fungal species was seen over the LDPE surface. LDPE with *Penicillium species* showed6.58% biogedradability as compared to4.65% with *Aspergillus fumigates* respectively. Initial and final dry weight of polythene was compared and percentage of degradation was calculated. *Penicillium species* was most effective in degrading LDPE than *Aspergillus fumigatus*.

Raaman, *et al.* (2012) isolated and identified low density polythene (LDPE) degrading fungal species from the polythene polluted sites at the Chennai. Isolated fungal species were identified as *Aspergillus terreus, A. flavus , A. niger, A. japonicas* and *Mucor species*. Predominant fungal strains *A. niger, A. japonicas* were selected for LDPE degradation due to their effectiveness over a period of 4 weeks in shaker culture under laboratory condition. Degradation of LDPE was measured in terms of mean weight loss, which was nearly 8 to 12% after a week. Further SEM was done at different magnification polythene strip show surface corrosion, folding and cracks and presence of porosity, fragility etc.

Sakhalkar *et al.* (2013) revealed the biodegradation of polythene (LDPE and HDPE) and Polyvinyl Chloride (PVC). Fungi with the ability to degrade plastics were isolated in synthetic medium supplemented with plastic powder or granules used for degradation study. Colonization study with the fungi showed a visible decrease in the polymer weight after 4-12 week incubation. Experiment of polythene degradation was done in two sets. In the first set fungi were isolated by using Synthetic Medium where glucose was used as carbon source. In this set about 15 fungal forms were observed. Out of 15 forms 6 forms were active. All these six forms with extensive network of fungal hyphae were

observed under light microscope. These forms were species of genus *Chrysonelia*, *Aspergillus*, *Penicillium*. In the second set of experiment plastic is used as sole carbon source about 13 different forms are found growing on the medium. Out of 13, 4 fungal strains were used for further studies. These forms were species of genus *Aspergillus*, *Penicillium, Fusarium* and *Chaetomium*.

Bhattacharyya, *et al.* (2013) isolated and identified the microorganisms from the soil during biodegradation testing of polyethylene and starch film. The isolation of microbial strain was carried out through soil serial dilution method and isolated microorganism was cultured in growth media. After growth of microorganisms at 37^0C they were identified as bacteria (*Pseudomonas species*, *Streptococcus species*, *Staphylococcus species*, *Micrococcus species* and *Moraxella species* etc), fungi (*Aspergillus niger*, *Aspergillus glaucus etc*), and *Actinomycetes* present on the surface of polyethylene and starch film. Surface morphology of polyethylene and starch film was analyzed by scanning electron microscopy (SEM) before and after degradation. Physico mechanical properties were also determined before and after degradation of film.

Nandi *et al.* (2013) studied the degradation of pretreated polythene with 99% of nitric acid incubated with fungi isolated from soil of municipal garbage. The fungi associated with degradation of polythene were identified by staining them with cotton blue. Efficiency of fungi in degradation of polythene was analyzed in shaker flask cultures in laboratory. The isolated fungi were analyzed for 2 to 8 months. The degradation of polythene ranged from 2.82 to 40.10%

.**Kumar *et al.* (2013)** studied the isolation of 60 marine bacteria from pelagic waters and then they were screened for their ability to degrade low density polythene. Among the 60 bacteria, 3 isolates were determined as polythene degrading. Isolated bacteria were identified on the basis of their 16S rRNA gene sequencing homology. The isolates M16 (accession no. KC355367), M27 (accession no. KC355368) and H1584 (accession no. KC355366) showed 99% homology with *Kocuria palastris*, *Bacillus pumilus* and 100% with *Bacillus subtilis* respectively. Polythene degradation in BH medium was initially monitored by the dry weight loss and after 30 days of incubation, the loss was 1.5% and 1.75% respectively. The maximum (32%) cell surface hydrophobicity was observed in M16, followed

by the H1584 and M27 isolates. The viability of the isolate growing on the polythene surface was confirmed using a tripheny tetra azolium chloride reduction test. The viability was also correlated with a concomitant increase in the protein density of the biomass. Polythene biodegradation was further confirmed by an increase in the Keto Carbonyl Bond Index which was calculated from FTIR.

Singh and Gupta (2014) reported the screening and identification of LDPE degrading fungi from polythene polluted sites. The fungal species were identified by plating and staining techniques. The screening of isolates was based on their ability to utilized LDPE as a primary carbon source. The screened fungi were identified as *Aspergillus flavus, A.niger, A.japonicus, Mucor, Fusarium species* and *Penicillium species*. Among the fungal strain, maximum degradation 36% was seen in *A.japonicus* , *Fusarium species* 32% and *A.flavus 30%* where as *Penicillium 24%, A.niger 20%, Mucor species* 16% degradation of LdPE was observed in 4 weeks of experiment in lab condition.

Dass and Kumar (2014) revealed that biodegradation of LDPE was achieved by fungal strains isolated from municipal solid waste. Efficacy of the microbes in polymer degradation was analyzed by weight reduction; change in pH, CO_2 evolution test over a period of 60 days at 30C.The colonization of fungal species on LDPE was seen by SEM whereas the surface chemical changes were confirmed by Fourier Transform Infrared Spectroscopy (FTIR). Isolated fungi were identified as *Aspergillus species* and *Fusarium species.*

Saminatham, *et al.* (2014) reported that the garden soil microbes were capable of degrading plastic material. The plastic samples taken in this study were polythene bag (A), plastic bag (B), plastic cup(C), and milk cover (D).Isolated strain was identified as *Pseudomonas putida* by performing appropriate identification test. Each plastic material was biodegraded with isolated strain of *P.putida* MTCC2475. It has been experimentally proved that *P.putida* cause degradation of all plastic samples used for this study such as A,B,C,D within a month in shaker culture . Among the plastic samples D (milk cover) was found 75% degradation.

Ambikadevi, *et al.*(2014) isolated bacteria from the marine water (Bay of Bengal).Isolation was done by the standard dilution plating technique on Zobella Marine Agar and Tryptone Soy Agar at 30^0C.

Low density polythene polythene (LDPE) and High density polythene (HDPE) polythene were used to check the degradability of bacterial strain. The degrading ability of the bacterial strains was evaluated by performing the Bacterial Adhesion to Hydrocarbon (BATH) test and Salt Aggregation Test (SAT). These tests are done for checking the hydrophobicity and colonization studies. Protein estimation was done over a period of one month for measuring the bacterial biomass in biofilms, colonizing the polythene surface. Polythene degradation was evaluated by weight loss of polythene and degradation was confirmed by SEM and FTIR analysis.

Sowmya, *et al.* (2014) reported bacteria *Bacillus cereus,* which was isolated from a local dumpsite of Shivamogga district degraded the polythene. Isolated bacteria were identified as gram positive rod by performing a biochemical test. Bacteria used polythene as carbon source for their growth. *Bacillus cereus* was grown on medium containing polythene and agar, after the growth on polythene it was screened for degradation of autoclaved; UV treated and surface sterilized polythene. *Bacillus cereus* was able to degrade UV treated polythene (14%) more efficiently than autoclaved (7.2%) and surface sterilized (2.4%). Degradation was monitored by weight, SEM and FTIR studies. Enzymes responsible for polythene degradation were identified as laccase and manganese peroxidase.

Bhatiya, *et al.* (2014) focused the degradation of LDPE by indigenous bacteria which was isolated from collected municipal waste. Biodegradation potential of bacteria was screened by growth enrichment medium containing 0.2gm LDPE powder. Screened bacteria were subjected to biodegradation of LDPE. Four bacterial strain degraded polythene 5%, 17.8%, 0.9% and 0.6% respectively. Degradation rate based on weight loss was analyzed through SEM and FTIR. Results illustrated one-step weight loss with control and three step weight loss with test. Isolated bacterial bacterial strain was identified by genomic DNA isolation followed by PCR and 16S RNA sequencing as *Pseudomonas citronellosis*. The isolate was named as *P. citronellosis* EMBS027 and sequence was deposited as LDPE degrading species, in GenBank with accession number KF361478.

International work on polythene degradation: Groups working in this field are listed as below-

Lee, *et al.* (1990) demonstrated the biodegradability of degradable plastic film containing prooxidant and starch under a pure shake flask culture system. Degradable plastic used in this study was produced commercially by Archer- Daniels- Midland POLYCLEAN. Three types of pretreatment procedure was used to check degradabity of polythene i.e. heat treatment at 70^0C, UV treatment with 365 nm and chemical disinfection of polythene. Known lignin-degrading bacteria *Steptomyces viridosporus* T7A, *S. badius* 252 and *S.setonii* 75Vi2 and fungus *Phanerochaete chrysosporium* were used. This was the first report demonstrating bacterial degradation of these oxidized polyethylenes in pure culture.

Bonhomme, *et al.* (2003) investigated the degradation of commercial environmentally degraded polythene in two stages. Degradable polythene was a green film (LC) containing TDPA. For this study a number of superimposed films were compression moulded at 100^0C to give sections of polymer. Firstly,biotic oxidation in an air oven to increase the effect of the compost environment and secondly in the presence of selected microorganism *(Nocardia asteroids, Rhodococcus rhodochrous, Cladosporium cladosporioides)*. Initial biofilm formation was identified by flourescence microscopy and the growth of bacteria was observed by SEM. It was observed that bacterial growth occurred on compression moulded polythene but had not been in preoxidized film. Molecular enlargement and broadening of molecular weight distribution occurred after preheating in air at 60^0C but not at an ambient temperature. Colonization of microorganism occurred on all samples. FTIR measurement was done for identification of degradation of film surface.

Loredo-Treviño, *et al.* (2011) worked on the isolation and characterization of fungal strains capable to use Polyurethane (PU) as sole carbon or nitrogen source describing the associated enzymes to PU biodegradation. In this work some fungi with capacity of growing with PU foam as nutrient source were isolated from sand contaminated with this plastic. Enzymatic activities of different fungi were determined in specific media as well as their invasion capacity on PU agar plate. 22 fungal strains demonstrated their capacity of growing on polyurethane. Among the enzymatic activities evaluated the most common was the urease activity (95% of the strains), protease 85%, esterase 50% and

laccase 36% respectively. Specific media used for enzyme estimation were YES medium for protease activity, Tween 80 for esterase activity, Christensen urea medium for urease activity and PDA-ABTS for laccase activity.

Ibibne, *et al.* (2013) studied polythene (LDPE&HDPE) biodegradation by *Bacillus mycoids* and *Bacillus subtilis (Bacillus species* indigenous to the Niger delta mangrove soil) was studied. HDPE and LDPE films were exposed to sunlight on the roof of a 4.5m tall building for 6 months between January and June. Exposure was performed according to ASTM standards using 45 angle wooden racks, facing south and then Polythene (PE) buried in mangrove soil after liming with $CaCO_3$ and fertilizer NH_4HPo_4. After 1, 2 and 3 month of burial, the PE pieces were removed and degradation was determined by measurement of the residual weight of the PE films and further biodegradation is confirmed by FTIR. Biodegradation in lab is done in Erlenmeyer flask by the bacteria in synthetic medium after 60 days of incubation. The result showed that *Bacillus mycoides* and *Bacillus subtilis* were capable of growing on PE films and biodegrade them after an initial abiotic degradation. Results of residual weight measurement revealed a dry weight loss ranging from 10.4% to 23.15% for LDPE and 8.41% to 17.72% for HDPE while the untreated films remained unchanged.

Yoon, *et al.* (2012) studied the isolation of strain capable of polythene degradation from a crude oil contaminated soil at room temperature. The isolated strain was rod-shaped gram negative bacteria identify as *Pseudomonas species*.E4 through the 16S rDNA sequencing technique. In previous studies preoxidised polythene used for the isolation of degrading strains, but in this study they used LMWPE, prepared by thermal degradation of a commercially available polythene under a nitrogen atmosphere. The average molecular weight (Mw) of LMWPE was in the range between 1700 and 23,700 indicated that 4.9-28.6% of the carbon was mineralized into CO_2 after 80 days at 37^0C. The alkane hydroxylase gene (alkB) of *Pseudomonas species* E4 was expressed in *E.coli* BL21 and the *recombinant E. coli* BL21 mineralized 19.3% of the carbon of LMWPE into CO_2 during the biodegradation in the compost at 37^0C for 80 days while the recipient cell was not active at all

towards the LMWPE biodegradation indicating that alkB from *Pseudomonas species* E4 played a central role in the LMWPE degradation.

Esmaeili, *et al*. (2014) reported the isolation of microorganism having potential to degrade Low density polythene (LDPE).Screening of degradation potential of microorganism experiment conducted by Enrichment procedure using LDPE powder as the sole source of carbon. Isolated microbial strains were identified as *Lycinibacillus xylanilyticus* XDB9 (T) strain S7-10F and *Aspergillus niger* strain F1-16S. The polythene degradation was performed for 56 days using UV irradiated pure LDPE film without pro-oxidant additives in a liquid mineral medium in the presence of bacterial isolate, fungal isolate and the mixture of the two isolate.

OBJECTIVES

The main emphasis of proposed work is to biodegrade the Low Density Polythene Bags (LDPE) in a relatively shorter duration. With this in view, following objectives are proposed

1. **To isolate, identify and screen microbial strains from different plastic waste dumpsites of Agra.**
2. **To study the biodegradation of polythene in vitro and in vivo condition by isolated microbial strain as well as with known microbial consortium.**
3. **To evaluate and optimize degrading ability of microorganism.**
4. **To study the effect of biodegradation on polythene.**

METHODOLOGY

Collections of soil sample will be done from different polythene waste dumping sites of Agra.

ISOLATION OF BACTERIA- Serial dilution technique would be used for the isolation of bacteria from soil sample and polythene inoculated on nutrient agar medium (Aneja 2001).

IDENTIFICATION OF BACTERIA- Identification of isolated bacterial strain will be performed on the basis of microscopic, macroscopy examination and biochemical tests. The bacterial isolates will be identified macroscopically by examining size, colony morphology; surface pigment, margin and microscopic examination including motility test and Gram staining.

Biochemical Test- Common biochemical tests conducted will be based on the keys detailed by Bergey's Mannual of Systematic Bacteriology (Clause and Berkely, 1986).

Catalase test: Place a microscope slide inside a petri dish. Keep the petri dish cover available.

Using a sterile inoculating loop or wooden applicator stick, collect a small amount of organism from a well-isolated 18- to 24-hour colony and place it onto the microscope slide. Using a dropper or pasteur pipette, place 1 drop of 3% H_2O_2 onto the organism on the microscope slide. Do not mix. Observe for the formation of bubbles against a dark background to enhance readability.

Oxidase Test: Soak a small piece of filter paper in 1% Kovács oxidase reagent and let dry. Use a loop and pick a well-isolated colony from a fresh (18- to 24-hour culture) bacterial plate and rub on to treated filter paper and observe for color changes. Microorganisms are oxidase positive when the color changes to dark purple within 5 to 10 seconds. Microorganisms are delayed oxidase positive when the color changes to purple within 60 to 90 seconds. Microorganisms are oxidase negative if the color does not change or it takes longer than 2 minutes.

Indole Test: Inoculate the tube of tryptone broth with a small amount of a pure culture. Incubate at 37°C for 24 to 48 hours.To test for indole production, add 5 drops of Kovác's reagent directly to the tube. A positive indole test will be indicated by the formation of a pink to red color ("cherryred

ring") in the reagent layer on top of the medium within seconds of adding the reagent. If a culture is indole negative, the reagent layer will remain yellow or be slightly cloudy.

Citrare test: Use a fresh (16- to 18-hour) pure culture as an inoculation source. Pick a single isolated colony and lightly streak the surface of the slant. A needle is the preferred sampling tool in order to limit the amount of cell material transferred to the agar slant. Avoid using liquid cultures as the inoculum source. Citrate utilization requires oxygen and thus screw caps, if used, should be placed loosely on the tube. Incubate at 35^{o}C (+/- 2^{o}C) for 18 to 48 hours. Some organisms may require up to 7 days of incubation due to their limited rate of growth on citrate medium.

Citrate positive: growth will be visible on the slant surface and the medium will be an intense prussian blue. The alkaline carbonates and bicarbonates produced as byproducts of citrate catabolism raise the pH of the medium to more than 7.6 causing the bromothymol blue to change from the original green color to blue Citrate negative: trace or no growth will be visible. No color change will occur; the medium will remain the deep forest green color of the uninoculated agar. Only bacteria that can utilize citrate as the sole carbon and energy source will be able to grow on the Simmons citrate medium, thus a citrate-negative test culture will be virtually indistinguishable from an uninoculated slant.

Urease Test: Use a heavy inoculum from an 18 to 24 hour pure culture to streak the entire slant surface. Do not stab the butt as it will serve as a color control. Incubate tubes with loosened caps at 35^{o}C.Observe the slant for a color change at 6 hours, 24 hours, and every day for up to 6 days. Urease production is indicated by a bright pink color on the slant that may extend into the butt. Any degree of pink is considered as a positive reaction. Prolonged incubation may result in a false-positive test due to hydrolysis of proteins in the medium. To eliminate protein hydrolysis as the cause of a positive test, a control medium lacking urea would be used.

Methyl red test: - Inoculate two MR-VP broths with the organism. Incubate at optimal temperature for 3-5 days. Add 3-4 drops of Methyl Red reagent to one tube. Interpretation for methyl red test-

Positive- Red color develops. Negative- Yellow color develops.

Carbohydrate fermentation test (Glucose, Lactose, Mannitol Maltose and Sucrose): Phenol Red will be used as an indicator in the media. At a neutral pH, the media is red; at a pH of less than 7, the media is yellow. Fermentation of the carbohydrate produces acid, causing the media to change from red to yellow.

Procedure: Inverted tube in broth, called Durham tube, will be used to capture some of the gas producing organism produces, allowing production to be seen (if it ferments, gas will be produced). Inoculate each media with the organism. Incubate at the optimum temperature for 24-48 hours.

Positive- Media turns yellow (fermentation has occurred) and gas produced.

Negative- Media remains red (no fermentation). Continue incubation of negative tubes for up to 2 weeks to detect slow fermenters.

Nitrate reduction: Boil and cool media with loose screw cap. Inoculate nitrate broth with the organism and incubate at optimal temperature for 2-8 hours. Add 5 drops of nitrate reagent A (sulphanilic acid) and add nitrate reagent B (dimethyl alpha naphthalamine) to the tube.

Positive- Red color indicates nitrate reduction to nitrite.

Negative- No color change.

ISOLATION OF FUNGI- Serial dilution technique would be used for the isolation of fungi from soil and polythene sample inoculated on Czapek dox agar medium.

IDENTIFICATION OF FUNGI- Isolated fungal strain will be identified by staining with lactophenol cotton blue as per the keys detailed by Gillman J.C. (1957) "A manual of soil fungi, 2nd edition the Iowa state university Press".

SCREENING OF POLYTHENE UTILIZING BACTERIA AND FUNGI- Polythene degrading microorganism will be screened by spread plate technique using mineral salt medium supplemented with 3% polythene powder. The composition of mineral salt media will be a

s follows: (g/l: K_2HPO_4 1.0, KH_2PO_4 0.2, NaCl 1.0, $CaCl_2.2H_2O$ 0.002, H_3BO_3 0.005, $NH_4(SO_4)_2$ 1.0, $MgSO_4.7H_2O$ 0.5, $CuSO_4.5H_2O$ 0.001, $ZnSO_4.H_2O$ 0.001, $MnSO_4.H_2O$ 0.001, $Fe_2(SO_4)_3.6H_2O$ 0.01, Agar 15) with pH 7.0. The plates will be incubated at 37^0 C for 7 d.

DEGRADATION OF POLYTHENE IN LAB: Shake Flask experiment or Liquid Culture Method

Procedure- The pre-weighed strip of LDPE films (1.5×1.5 cm) will be aseptically transferred into the conical flask containing 300 ml of mineral salt medium (broth) and then inoculated with identified polythene degrading microorganisms. Control will be maintained with LDPE films in the microbe free medium and left in a rotary shaker at 30°C, 150 rpm for 1,2,3,4 or 5 month. after the period of shaking the strips will be collected washed using distilled water, dried in shade and further analyzed for biodeterioration.

DEGRADATION OF POLYTHENE IN SOIL: Soil Burial Test

Procedure-The soil burial test will also be carried out for evaluating the biodegradability of the blends. The soil will be taken in pots and the plastic strips will be placed in it. The bacterial culture will be supplied to the soil. Care will be taken to ensure that the samples were completely covered with soil. The pot will be kept at room temperature. The loss in weight and tensile strength will be measured after thorough washing with water and drying in oven.

EVALUATION OF DEGRADING ABILITY OF MICROORGANISM BY

BATH test-Bacterial cell-surface hydrophobicity will be estimated by the bacterial adhesion to hydrocarbon (BATH) test was proposed by Rosenberg (1980) and is based on the affinity of bacterial cells for an organic hydrocarbon such as hexadecane. For the BATH test, bacteria will be cultured in NB medium until the mid exponential phase, centrifuged and washed twice with phosphate–urea–magnesium (PUM) buffer containing (g l-1): K_2HPO_4, 17; KH_2PO_4, 7.26; urea, 1.8 and $MgSO_4.7H_2O$, 0.2. The washed cells will be resuspended in PUM buffer to an O.D. (400 nm) value of 1.0–1.2. Aliquots (1.2 ml each) of this suspension were transferred to a set of test tubes, to which increasing volumes (ranging: 0–0.2 ml) of hexadecane were added. The test tubes were shaken for 10 min and then allowed to stand for 2 min to facilitate phase separation. The turbidity of the aqueous suspensions will be measured at O.D. 400 nm. Cell-free buffer served as the blank.

SAT test-This test proposed by Lindahl (1981). Bacterial suspensions 10 micro liters will be mixed with equal volumes of ammonium sulphate solutions of various molarities (0.2M-4M) on a glass slide and observed for aggregation for 1 minute at room temperature. The highest dilution of ammonium sulphate giving visible aggregation will be scored as a numerical value for surface hydrophobicity, the SAT value.

EFFECT OF DEGRADATION ON POLYTHENE- Biodegradation of polythene will be analyzed by weight reduction, change in pH of medium, strum test and by using Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR).

Weight reduction: A simple way to measure the biodegradation of polymers is by determining the weight loss. To facilitate accurate measurement of the dry weight of the polyethylene, the fungal colonization will washed off from the polymer surface by dipping the fungi treated films with 2 % (v/v) aqueous sodium dodecyl sulphate solution for 4 h and then with distilled water. The washed polymer film will be placed on a filter paper and dried overnight at 60^0C before weighing. The percentage of weight loss was calculated by the following formula-

$$\%\ weight\ loss = \frac{initial\ weight - final\ weight}{Initial\ weight\ x\ 100}$$

SEM Analysis: The fungal attachment of surface films and different changes on surface of polythene such as micro cracks pits on LDPE film by growing microorganism will be visualized by SEM.

FTIR Analysis: Fourier Transform Infrared analysis will be carried out for identification of surface structural changes on polythene surface.

Sturm test:

100 ml capacity autoclaved plastic containers will be used for the study. Separate setup was maintained with uninoculated SM supplemented with LDPE powder. After the stipulated time (48h) the KOH solution (1 M) that had trapped the CO_2 liberated by the inoculant (after utilization of LDPE the sole carbon source) will be gravimetrically quantified using barium chloride. The dissolved carbon dioxide present in the medium will be also estimated using titration method.

Briefly, sample (25 ml) will be taken in a conical flask and 0.05 ml of 0.1 N Thiosulphate solution was added. After the addition of 2 drops of methyl orange indicator, this solution will be titrated against 0.02 Sodium Hydroxide solutions. End point will be the change in color from orange red to yellow. Following this, two drops of phenolphthalein indicator will be added and titration continued till pink colors develop. Volumes of the titrant used will be noted and the amount of CO_2 calculated.

SIGNIFICANCE

Polythene are unavoidable face of our daily life necessities but at the same time it also brings long terms harmful effect on flora, fauna and environment. Utility of polythene is increasing at 12% per year in world posing threat to global ecology. This uncontrolled and frequently deliberate release of polythene is major cause of growing environmental pollution. Disposal of plastic is very big challenge to researchers because generally the method used for disposal, causes secondary problem such as incineration cause air pollution, landfill cause soil pollution and ground water pollution and recycling is not cost effective. The biodegradation of polythene through microbes is low cost, low technology and eco friendly treatment, capable of reducing and even eliminating plastic waste solution.

References

Ambikadevi, K, P V Ratnasari, B K M Lakshmi, and K P J Hemalatha. "Isolation of polythene degrading bacteria from marine waters of Viskhapatnam, India." *International journal of Current Microbiology and Applied Sciences* 3, no. 10 (2014): 269-283.

Aneja, K R. *Experiment in Microbiology Plant pathology Tissue Culture and Mushroom Production Technology.* 3. New Delhi: New Age International(P)Ltd, 2001.

Arkatakar, M, A Artutchelvi, J Sudharkar, M Bhaduri, S Uppara, and P V Doble. "Approaches to Enhance the Biodegradation of Polyolefins." *The open Envinonmental Engineering Journal* 2 (2009): 68-80.

Astrup, T, T Moller, and J Fruergaard. "Incineration & Co-combustion of waste Accounting of greenhouse gases & global warming contribution." *Waste Management* 27 (2009): 789-799.

Balasubramanian, V, K Natrajan, B Hemambika, B Ramesh, and C S Sumathi. "High- density polythene (HDPE)-degrading potential bacteria from marine ecosystem of Gulf of mannar, India." *letters in Applied microbiology* 51 (2010): 205-211.

Bharwaj, H, R Gupta, and A Tiwari. "Microbial Population Associated With Plastic degradation." *Open Access Scientific Reports* 1, no. 5 (2012): 272-274.

Bhatiya, M, A Girdhar, A Tiwari, and A Nayarisseri. "Implication of a novel Pseudomoonas species on law density polyethylene biodegradation: An in vitro to in silico approach." *Springer* 3, no. 1 (2014): 1-10.

Bhattacharyya, S, V Verma, R K Kalyan, V Kumar, K N Panday, and P Sharma. "Studies on Isolation and Identification of Active Microorganeisms during Degradation of Polythylene/ Starch film." *International Research Journal of Environment Science* 2, no. 9 (2013): 83-85.

Bonhomme, S, A Cuer, A M Delort, J Lemaire, M Sancelme, and G Scott. "Environmental biodegradation of polythene." *Polymer Degradation and Stability* 81 (2003): 441-452.

*Brydson, J. *Plastics Materials ISBN 0-7506-4132.* 7 . Elsevier, 1999.

Chandrakar, M Bhatiya, A Girdhar, and A Tiwari. "Implicating Nanoparticles as Potential Biodegradation Enhancer." *Nanomedicine & Nanotechnology* 4, no. 4 (2013): 1-7.

*Crystal, M. "Harmful Effects of Plastic Waste Disposal." *e How (WEb),* 2014.

Dass, M P, and S Kumar. "Micrensity Deteriotion of Low Density Polythylene by Aspergillus and Fusarium sp." *International Journal of Chem Tech Research* 6 (2014): 299-305.

*Derraik, JG B. "the pollution of the marine environment by plastic debris: a rewiew." *Marine Pollution Bulletin* 44 (2002): 842-852.

El-Shafei, M, H A El-nasser, and A L Ali. "Biodegradation of disposable polythene by fungi and Streptomyces species." *Polymer Degradation and Stability* 62 (1998): 361-365.

Esmaeili, A, A A Pourbabaee, H A Alikhani, F Shabani, and L Kumar. "Colonization and Biodegradation of Photo-Oxidized low-Density Polyethylene(LDPE) by New Strains of Aspergillus sp. and Lysinibacillus sp." *Bioremediation Journal* 18 (2014): 213-226.

Fontella, S, S Bonhomme, M Kounty, L Husarova, and JM Brusson. "Comparison of the biodegradability of various polythene films containing pre-oxidant Additives." *Polymer Degradation Stability* 95 (2009): 1011-1025.

Fourst, C J, R R Mills, T Hass, R Castevans, and R Ottenbrite. "Biodegradation of LDPE/Cellulose blends by common fungi." *Macromol. Symp.* 123 (1997): 1-8.

G., Swift. *Non Medicle biodegradable polymers: Environmentally degradable polymer, In J. Domb, J. Kost< and Wiseman, cds. Handbook of Biodegradable Polymers.* Amsterdam, 1997.

Gilan(Orr), Y Hadar, and A Sivan. "Colonization , biofilm formation and biodegradation of polythene by a strain of Rhodococcus ruber." *Applied microbial and cell physiology* 65 (2004): 97-104.

Hadad, A, D Geresh, and S Sivan. "Biodegradation of Polythene by thermophilic bacterium Brevibacillus borstelensis." *Journal of Applied Microbiology* 98 (2005): 1093-1100.

Ibibne, A A, H O Stanley, and O M Immunel. "Biodegradation of Polythylene by Bacillus sp. Indigenous to the Niger Delta Mangrove Swamp." *Nigerian Journal of Biotechnology* 26 (2013): 68-79.

Kapri, R, A Zaidi, and M G Goel. "Implication of Spion and NBT nanoparticles upon in-vitro and in-situ biodegradation of LDPE film." *Micribial Biotechnology* 20 (2010): 1032-1041.

Karpi, R, A Zaidi, and M G H Goej. "Nanobarium titanate as supplement to accelerate plastic waste biodegradation by indigenous bacterial consortia." *AIP Conf Proc* 1147 (2009): 469-474.

Kathiresan, K. "Polythene and Plastic-degrading microbes from the mangrove soil." *Centre of Advanced Stydy in Marine Biology* 51, no. 3 (2003): 629-63.

Koutny M, Lemaire J, Delort AM. "Biogedradation of polyethylene films with prooxidant additives." *Chemosphere* 64 (2006): 1243-1252.

Kumar, H, and B Jha. "Biodegradation of low-density polyethylene by marine bacteria from." *ELsavier* 77 (2013): 100-106.

Kyaw, BM, R Champakalakshmi, CS Lim, MK Sakharkar, and KR Sakharkar. "Biodegradation of Loe Density Polythene (LDPE) by Pseudomonas species ." *Indian ournal of Microbiology*, 2012.

Lee, B, A L Pometto, A Fratzke, and T B Bailey. "Biodegradation of Degradable Plastic Polythene by Phanerochaete and Streptomyces Species." *American Society for Microbiology* 57 (1990): 678-685.

Lindahl, M, A Faris, T Wadstrom, and S Hjerton. "A new test based on "salting out" to measure relative surface hydrophobicity of bacterial cells ." *Biochim. Biophys. Acta* 77 (1981): 471-476.

Loredo-Treviño, A, G García, A Velasco-Téllez, A Rodríguez-Herrera, and A C Aguilar. "Polyurethane foam as substrate for fungal strains." *Advances in Bioscience and Biotechnology* 2 (2011): 52-58.

Mahalaxmi, V, A Siddiq, and S N Andrew. "Analysis of Polythene degrading potential of Microorganism Isolated From Compost Soil." *International Journal of Pharmaceutical & Biological Archives* 3, no. 5 (2012): 1190-1196.

* Myer, Kutz. *Handbook of Material Selection John Wiley & Sons ISBN 0-44471-5924-6.* 2002.

Nanda, S, J Abraham, and S S Sahu. "Studies on the biodegradation of natural and synthetic polyethylene by Pseudomonas." *J. Appl. Sci. Environ. Manage* 14, no. 2 (2010): 57-60.

Nandi, R G, and M Joshi. "Biodegradation of pre treated polythene by different species of Aspergillus isolated from garbage soil." *Microbiological Communication* 6, no. 2 (2013): 199-201.

Pommetto, A L, B T Lee, and K E Jhonson. "Production of an extracellular polythene degrading enzymes by Streptomyces Species." *Applied Environmental Microbiology* 58 (1992): 731-733.

Pramila, R, and Vijaya K Ramesh. "Biodegradation of low density polythene (LDPE) by fungi isolated from marine water- a SEM analysis." *African journal of Microbiology Research* 5, no. 28 (2011): 5013-5018.

Pramilla, R, R Padamvathy, K V Ramesh, and K Mahalaxmi. "Brevibacillus parabrevis, Acinetobacter baumannii and Pseudimonas citronellolis- Potential candidates for biodegradation of loe density polythene (LDPE)." *Journal of Bacteriology Research* 4, no. 1 (2012): 9-14.

Premraj, R, and M Dobley. "Biodegradation of Polymer." *Industrial Journal of Biotechnology* 4 (2005): 186-193.

Raaman, R, N Rajitha, A Jayshree, and R Jegadeesh. "Biodegradation of plastic by Aspergillus spp. isolated from polythene polluted sites around Chennai." *Acad Indus Res* 1, no. 6 (2012): 313-316.

Rajandas, H, S Parimannan, K Sathasivam, and LS Yin. "Novel FTIR-ATR spectroscopy based technique for the estimation of lowdensity polythene biodegradation." *Polym Test,* 2012.

Rivard, S, C Moens, L Roberts, K Brigham, and J Kelley. "Starch ester as biodegradable plastic: Effects of ester group chain length and degree of substitution on aerobic biodegradation." *Enzyme Microbiology Technology* 17 (1995): 848-852.

Rosenberg, M, D Gutnik, and E Rosenberg. "Adherence of bacteria to hydrocarbons: a simple method for measuring cell surface hydrophobicity." *FEMS Microbiology Letter* 9 (1980): 29-33.

Sakhalkar, S, and K L Mishra. "Screening and Identification of Soil Fungi with Potential of Plastic Degradation." *Indian Journal of Applied Research* 3, no. 12 (2013): 62-64.

Saminatham, P, A Sripriya, K Nalni, T Shivkumar, and V Thangapandian. "Biodegradation of Plastics by Pseudomonas putida isolated from Garden Soil Samples." *Journal of Advanced Botany and Zoology* 1, no. 3 (2014): 1-4.

Scott, G. *Polymer in modern life. In: polymer and Environment.* UK: THE royal Society of Chemistry, Cambridge, 1999.

Secchi, E R, and S Zuzur. "Plastic debris ingested by a Blainville's beaked whale, Mesoplodon densirostris, Washed ashore in Brazil." *Auatic Mammal* 25 (1999): 21-2.

Sepulveda, T V, E F Torres, A M Guzman, M L Gonzalez, and G T Quintero. "Microbial degradation of thermo-oxidised low density polyethylene." *Journal of Applied Polymer Science* 73 (1999): 1435-1440.

Sindhuja, P, M Padmapriya, R Pramila, and R K Vijaya. "Biodegradation of Loe Density Polythene (LDPE) by Fungi isolated from marine water." *Research Journal of Biological Sciences* 6 (2011): 141-145.

Singh, J, and K C Gupta. "Screening and Identification of Low Density Polythythene (LDPE) Degrading Soil Fungi Isolated from Polythene polluted Sites around Gwalior city(M.P)." *International Journal of Current Microbiology and Applied Sciences* 3 (2014): 443-48.

Singh, V, M Dubey, and S Bhadauria. "Microbial Degradation of Polythene(Low Density) By Aspergillus Fumigatus and Penicillium Sp." *Society of Applied Sciences* 3, no. 3 (2012): 498-501.

Sivan, A. "New perspective in plastic biodegradation." *Current Opinion in Biotechnology* 22 (2011): 422-426.

Sowmya, H V, Ramlingappa, M Krishnappa, and B Thippeswamy. "Biodegradation of Polythene by Bacillus cereus." *Advances in Polymer science and Technology* 4, no. 2 (2014): 28-32.

Steven, S E. *Green plastics: an introduction to the new science of biodegradable plastic.* Princeton University Press, 2002.

Sudhakar, M, M Doble, P S Murthy, and R Venkatesan. "Marine microbe mediated biodegradation of low and high density polyethylenes plastics." *International Biodeterioration and Biodegradation* 61 (2008): 203-213.

Tensel, B, and B Z Yildiz. "Goal based waste management strategy to reduce persistence of contaminant in leachate at municipal solid waste landfills." *Environment Dev. Sustain.* 13 (2011): 821-831.

Valanvannidis, K, A Iliopoulos, N Gotsis, and G Fiotabis. "Persistent free-radicals, heavy metals and PAHs generated in partoculate soot emissions and residue ash from controlled combustion of common types of plastic." *Hazard Mater* 156 (2008): 277-284.

Vijaya, Ch, and R M Reddy. "Impact of soil using municipal solid waste on biodegradation of plastic." *Indian Journal of Biotechnology* 7 (2007): 235-239.

Watanabe, T, Y Ohtake, H Asabe, N Murakami, and N Furukawa. "Biodegradability and degrading microbes of low density polyethylene." *Applied Polymer Science* 111 (2009): 551-559.

* Weiland, M, A Daro, and C David. "Biodegradation of thermally oxidised polyethylene." *Polymer Degradation Stability* 48 (1995): 275-289.

Yamada, O K, H Mukumoto, Y Katsuyaya, A Saiganji, and Y Tani. " Degradation of polyethylene by a fungus Penicillium simplicissium YK." *Polymer Degradation Stability* 72 (2001): 323-32.

Yoon, M G, H J Jeon, and M N Kim. "Biodegradation of Polythene by a soil Bacterium and AlkB Cloned Recombinant cell." *Bioremediation and Biodegradation* 3, no. 4 (2012): 1-8.

*Zhang, Z, J Wang, X Gang, and J Gu. "thalateA study on the biodegradability of polythene terephthalate fiber and diethylene glycol tereph." *Applied polymer science* 93 (2004): 1089-1096.

* Original paper not seen